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Reclassification of *Eimeria schmidti* Al-Yousif *et al.* (1997) (Apicomplexa: Eimeriidae) with Description of its Endogenous Stages

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Abstract.- *Eimeria schmidti* was previously described as a new species found in the gallbladder of sandy fringed-toed lizards *Acanthodactylus schmidti* in Saudi Arabia. According to the oocyst morphology, site of infection and endogenous development, the coccidium was transferred to genus *Choleoeimeria* and renamed *Choleoeimeria schmidti*. Meronts, gamonts and young oocysts were detected and described at light and electron-microscopic level. Merozoites with a large number of micronemes and long rhopteries were observed and their nuclei were seen located posteriorly. Microgamonts contained many nuclei at their margins. These nuclei had homogenate cytoplasm and did not likely have nucleoli. Macrogamonts were recognized by their nuclei each with a nucleolus and characterized by wall-forming bodies 1 and 2. All stages contained electron–dense inclusions and vacuoles, which appeared to be one of the characteristic features of *Choleoeimeria.*

Key words: Coccidia, *Choleoeimeria*, *Acanthodactylus*, gall bladder, Saudi Arabia.

INTRODUCTION

Proposed replacement of reptilian *Eimeria* species in a separate genus referred to as *Choleoeimeria* (Paperna and Landsberg, 1989a). Their proposal was based on the restricted growth of endogenous stages to the gallbladder epithelium, hypertrophy of the infected host cells and their protrusion into the bile lumen and production of ellipsoidal to cylindrical oocysts, which sporulate endogenously while still in the gall bladder. This classification was rejected by a number of authors (Telford, 1992, 1998; Upton *et al*., 1993; Maupin *et al*., 1998; Modry *et al*., 2000, 2001; Slapeta *et al*., 2003). However the generic separation was confirmed by Jirku *et al*. (2002), via molecular tools (PCR). Many species of *Choleoeimeria* were recognized from different reptilian families including Geckonidae, Chameleonidae, Scincidae, Iguanidae and Teiidae and several snakes (Lainson and Paperna, 1999; Sloboda and Modry, 2006; Paperna, 2007a; Abdel-Baki *et al*., 2008). Paperna (2007a) stated that many eimerine species known to develop in the gallbladder epithelium have been reported in literature, their generic status should be amended. In a previous study, a tetrasporocystic, dizoic, coccidian oocysts were described from the gallbladder of *Acanthodactylus schmidti* and the parasite was named *Eimeria schmidti* Al-Yousif *et al*. (1997). Their description was only limited to the exogenous stages and the site of infection, but nothing was reported about the endogenous development. However, for allocating a coccidian parasite to its correct genus and species, it is fundamental to follow structure and mode of development of its endogenous stages. Therefore, it was necessary, through this study to reexamine the exogenous and endogenous stages of the parasite using light and electron microscopy and correct its systematic position.

MATERIALS AND METHODS

A total of 61 sandy fringed-toed lizards (*Acanthodactylus schmidti*) were collected from Al-Qassim Region, Saudi Arabia, during 2008, and transferred to the laboratories of Faculty of Sciences and Arts, Qassim University. Lizards were kept separately in plastic cages for several hours to obtain faeces. Microscopic examination of a suspension of faeces, macerated in water, revealed the presence of coccidian oocysts. The infected animals were killed with chloroform and dissected. Bile was collected with finely pointed glass pipette. Squash preparations of gallbladder wall, bile and

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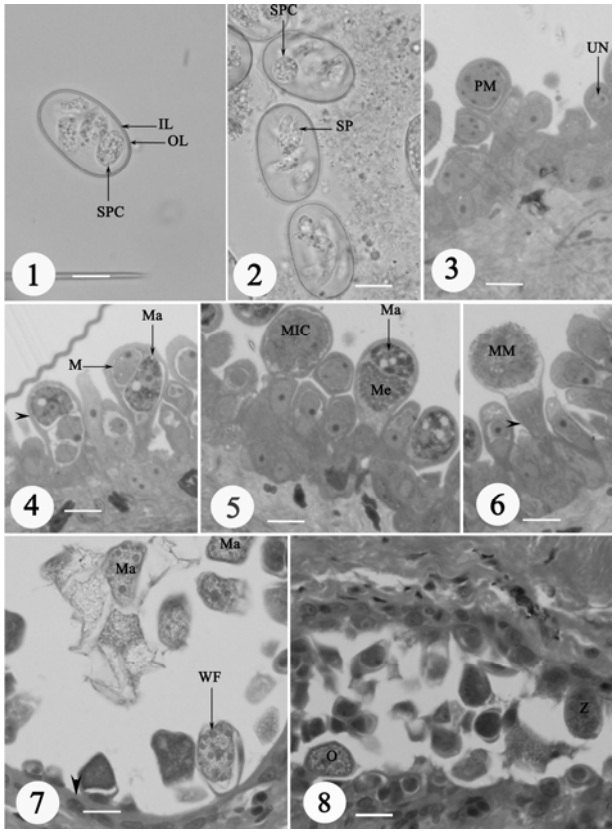
selected parts of the alimentary canal were examined with a light microscope for the presence of the parasites.

To study the endogenous stages, the gallbladder wall was fixed in 10 % buffered formalin, processed for histology, sectioned, stained with haematoxylin and eosin, examined and photographed with a Zeiss photomicroscope. For electron microscopy, small pieces of gallbladder were fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1M, pH 7.4) for 24 hours at 4 ˚C and rinsed repeatedly in the same buffer. post-fixation, for one hour, was carried out by osmium tetroxide, rinsing in buffer, dehydrated in graded ethyl alcohol and embedded in Agar 100 medium (Agar Scientific Ltd., U.K). Reichert Ultracut with glass knives was used to obtain semi and ultra-thin sections. The semi-thin sections were examined and photographed with Zeiss 100 photomicroscope, while ultra-thin ones were stained on grids with uranyl acetate and lead citrate and examined by Joel 100 CX transmission E. M. All measurements were given in micrometers (µm).

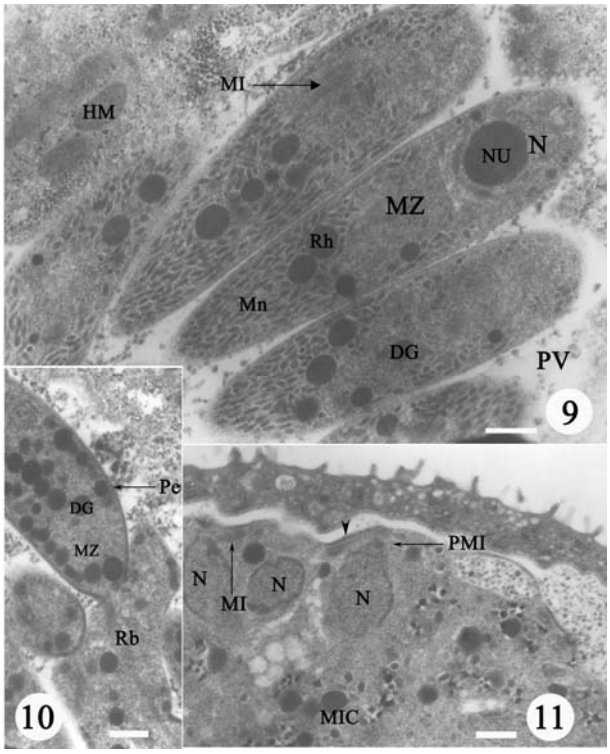
RESULTS

Light microscopy

The percentage of infection of lizard with *Choleoeimeria schmidti* was 34.4% (21/61). Oocysts obtained from faeces were ellipsoidal in shape and sporulated, each with four sporocysts, each sporocyst contained two banana–shaped sporozoites. Sporulated oocysts measured 31 x 20 (32 –28 x 21 -17). The length/width ratio (L/W) was 1.55 (1.5 - 1.6). Oocyst wall was double–layered, greenish yellow, smooth and without striations and measured 1.3 (0.9– 1.4) thick (Fig. 1). Sporulation occurred in the gallbladder. Oocyst residuum, micropyle, and polar granules were absent. Ellipsoidal Sporosysts appeared without Steida and substeida bodies or other localized thickenings of their walls and measured 10.5 x 8 (9-12 x 6-8.5) with shape index 1.3 (1.2-1.6) (Figs. 1, 2). Head to tail sporozoites were longitudinally located and curved around the granular sporocyst residuum, measured 11 x 2 (10.8 -11.3 x 1.8 - 2.2) and each with conspicuous anterior and posterior refractile bodies (Fig. 2).



Figs. 1-8. Various exogenous and endogenous stages of *Choleoeimeria schmidti* Alyousif *et al.* (1997) comb. nov. infecting the gallbladder of *Acanthodactylus schmidti.* All scale bars = 10 µm. 1, mature oocyst surrounded by outer layer (OL) and inner layer (IL) membrane and containing four sporocysts (SPC); 2, mature oocysts containing sporocysts (SPC) and free sporozoites (SP); 3, uninucleated (UN) and premature meront (PM); 4, infection of epithelial cell by two parasites, meront (M) and macrogamont (Ma), the host cell is displaced into the lumen but still attached to the underlying epithelium via a stalk–like structure. Host cell infected with three parasites (arrowhead); 5, microgamont (MIC), macrogamont (Ma) and mature meronts (Me); 6, mature microgamonts (MM), attached to the underlying epithelium via a stalk–like structure (arrowhead), shedding microgametes, note disappearance of the parasite membrane and fragmentation of its surface; 7, macrogamontes (Ma) with wall-forming bodies (WF). Note that the epithelium remained single-layered (arrowhead); 8, zygotes (Z) and fully-formed oocysts (O).



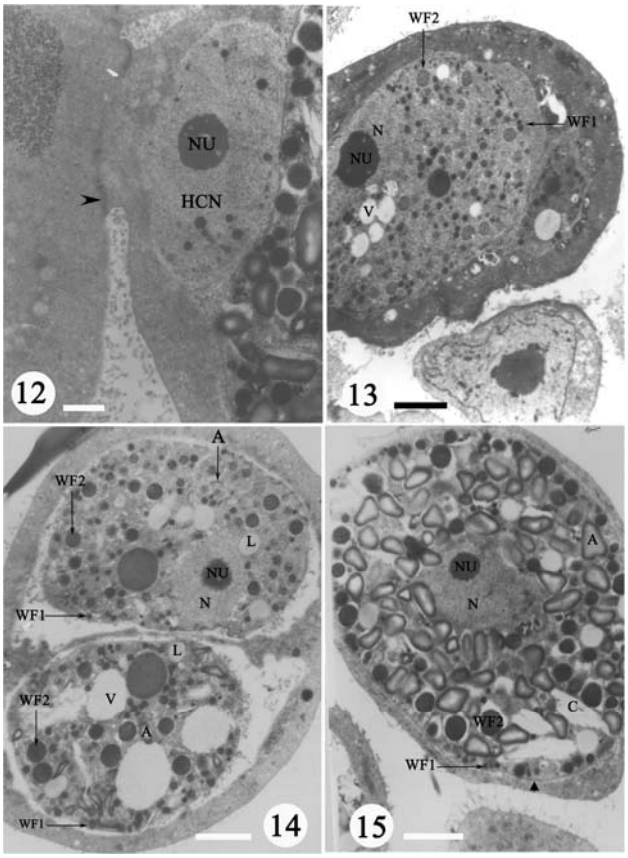
Figs. 9-11. Electron micrographs of the endogenous stages of *Choleoeimeria schmidti*. 9, merozoites, obliquely–cut in a parasitophorus vacuole (PV). Each merozoite (MZ) has micronemes (MN), dense granules (DG), a posteriorly–located nucleus (N) with a nucleolus (NU) and Mitochondrion (MI) Scale bar: 500 nm; 10, emergence of merozoite (MZ) from the residual body (Rb), each merozoite is surrounded by a double-membrane pellicle (Pe). Note the presence of large number of dense granules (DG). Scale bar: 500 nm; 11, microgamont (MIC) with peripherally arranged nuclei (N), without nucleoli, note the presence of mitochondrion (MI) and centriole (C) in the vicinity of the nuclei. PMI- emerging microgamete. newly–formed microgametes are surrounded by pellicle (arrowhead). Scale bar: 500 nm.

Endogenous development of the detected parasite was observed in the gallbladder epithelium of *Acanthodactylus schmidti.* The infected cells were hypertrophied and emerged over the neighboring cells into the gallbladder lumen, developing stalk–like structures through which they remain attached to the underlying epithelium, constituting a stratified epithelium (Figs. 3-6, 8). In some lower level of infections, the epithelium remained single–layered (Fig. 7). Host cells usually contained one parasite, exceptionally two or three, some mature meronts or gamonts were accompanied by a second young stage beneath them (Figs. 4, 5). Young meronts measured 3.5 – 4.2 x 3 – 3.5 (Fig. 3), mature ones were 15.1 - 15.4 x 14.2 - 14.4 in size in cross section. Meronts were estimated to produce up to 25 merozoites (Fig. 5). Microgamonts with peripherally arranged nuclei measured approximately 20.3 - 19.5 x 15.5 -16.5 and were estimated to produce over 50 microgametes per section (Fig. 6). Mature macrogamonts with wall-forming bodies measured 13.8 – 14.2 x 10.6 – 11.2 (Fig. 7). Young oocysts (zygotes), filled with amylopectin granules, were 15.9 - 16.2 x10.3 - 11.2 in size (Fig. 8).

Ultrastructure

Each parasitophorous vacuole of mature meront contained dispersed electron–dense small structures as well as a large number of merozoites which were cut in different positions (Fig. 9). Merozoites were banana-shaped and located in a parasitophorous vacuole in the hypertrophied, displaced host cell (Fig. 9). They were surrounded by a pellicle (Fig. 10). The anterior third of the merozoite was filled with a large number of the electron-dense small micronemes, followed, in behind, by a few number of large-size rhoptries of the same electron density (Fig. 9). Few rhoptries were seen with their long ducts. It was noticed that merogony is exopolygenous, with a remaining residual body (Fig. 10). Newly formed merozoites contained a large number of electron-denese granules (may be rhopteries) than mature ones (Fig. 10). Merozoites nuclei were posteriorly located, each with a large electron–dense nucleolus (Fig. 9).

Microgamonts were characterized by peripherally arranged nuclei, with homogenous nucleoplasm and lacked nucleoli (Fig. 11). In the vicinity of each nucleus, a pair of centrioles was seen beneath the parasite cell membrane. Microgamonts harbored many electron–dense granules, vacuoles as well as mitochondrial aggregates.



Figs. 12-15. Electron micrographs of bile cells infected with different developmental stages of macrogamont. 12, the junction (arrowhead) between a displaced, infected and hypertrophied epithelial cell and the underling cell, host cell nucleus (HCN) and nucleolus (NU). Scale bar = 1µm; 13, young macrogamont with wall- forming bodies 1 (WF1) and 2 (WF2) and vacuoles (V). N- nucleus, NU- nucleolus. Scale bar: 2 µm; 14, a host cell parasitized by two macrogamonts. Note the presence of nucleus (N), nucleolus (NU), lipids (L), amylopectin (A), vacuole (V), wall forming bodies 1 (WF1) and 2 (WF2). Scale bar : 2 µm; 15, mature macrogamete full of amylopectin granules (A) and canaliculi (C). Wall-forming bodies 1 (WF1) and 2 (WF2) still remaining. Note the thickening of some parts of macrogamete membrane (arrowhead), Nucleus (N) and nucleolus (NU). Scale bar: 2 µm.

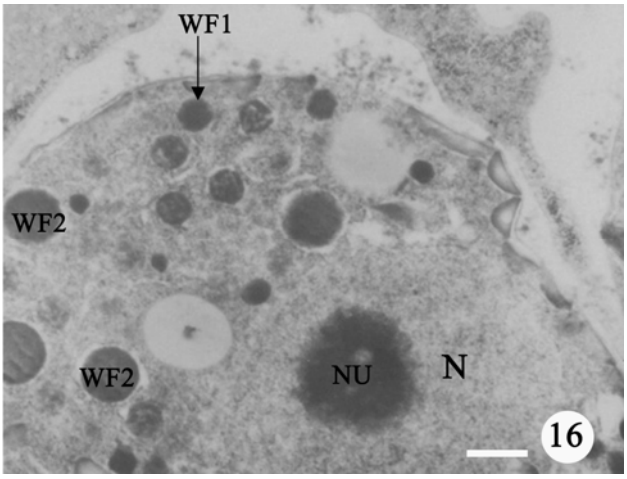


Fig. 16. Electron micrograph of macrogamete with wall-forming bodies 1(WF1) & 2 (WF2). Scale bar : 500 nm

Young macrogamonts were surrounded by a parasitophorous vacuole and filled with small granules and large spherical structures surrounded by a single unit membrane. They were distinguished by the presence of two types of the wall-forming bodies 1 & 2 and lodged nuclei. Each nucleus contained a large electron–dense nucleolus. The wall–forming bodies 1 were small and numerous while the wall–forming bodies 2 were larger in size, fewer in number and surrounded by a cisterna of endoplasmic reticulum (Figs. 13,16).

Mature macrogamonts and zygotes were characterized by a huge number of amylopectin granules scattered in their cytoplasm together with less number of lipid vacuoles and numerous canaliculi. Wall-forming bodies 1 nearly disappeared but scarce number of wall–forming bodies 2 were still present (Figs. 14, 15). As the development of macrogamonts proceeded, their membranes became more electron dense in some parts suspected to be the precursors of the oocyst wall.

The parasitophorous vacuole contained a lot of electron–dense, sparse, small inclusions, and was surrounded by many host–cell mitochondria (Figs. 14 – 16). The host–cell nuclei suffered hypertrophy and malformation in shape by having twisted membranes (Fig. 12).

DISCUSSION

Lainson and Paperna (1999) stated that it is impossible to be sure of the generic status of many reptilian eimeriids without reference to the endogenous stages. Unfortunately, this has not been done in many descriptions of such parasites. In the present work, we found that the features of *E. schmidti*, previously described by Al- Yousif *et al*. (1997) as a new species infecting the gallbladder of sandy fringed-toed lizards *Acanthodactylus schmidti*, coincide with those mentioned by (Paperna and Landsberg, 1989a). Therefore, we reclassified *E. schmidti* as a member of the genus *Choleoeimeria*.

Histological features of the endogenous development for the following species *C. alloagamae*; *C. xiang-maii*; *C. allogehyrae; C. boulii; C. heteronotis; C. calotesi; C. lygosomis* and *C. sylvatica* (Paperna, 2007a); *C. carinii* and *C. rochalimai* (Lainson and Paperna, 1999) were previously described from different reptilian hosts. In addition to the current species, The endogenous development of all the detected *Choleoeimeria* species were restricted to the gallbladder epithelium of reptiles in contrast to *Eimeria* which have a wide range of infection (Paperna, 1993). In the present study it was observed that the infected host cell was greatly hypertrophied, became stratified, enlarged, and protruded into the bile lumen but remained attached to the underlying epithelium via a narrow stalk. The same observations were recorded in *C. alloagamae*; *C. xia-maii* but not in *C. allogehyrae; C. boulii* and *C. heteronotis* which were described as a single layer. *C. schmidti* differs from *C. alloagamae*; *C. allogehyrae*; *C. calotesi* and *C. heteronotis* in having smaller mature meronts. Macrogamonts are smaller than those described in *C. heteronotis* but larger than those described in *C. alloagamae* and *C. calotesi*. Microgamonts are smaller than those described in *C. heteronotis* but larger than *C. allogehyrae*.

Only few ultrastructural studies have been reported as *C. turcicus*, parasitizing *Hemidactylus tucicus* from Israel (Paperna and Landsberg, 1989b); *C. rochalimai* from *H. mabouia* from north Brazil (Paperna and Lainson, 2000); *C. alloagamae* from *agama sp*., from Cameroon; *C. allogehyrae* from *Gehyra austrialis*, from Australia; *C. heteronotis* from *Heteronotia binoei* from Australia and *C. pachydactyli* from *Pachydactylus capensis* from Republic of South Africa (Paperna, 2007b). In the present study, it has been noticed that merozoites emerged into parasitophorous vacuoles and were bounded by a double–layered pellicle, which coincide with those described in *C. alloagamae*. However, in this work a residual body was seen and the ducts of rhoptries seemed to be longer than those recorded from other *Choleoeimeria* species (Paperna, 2007b). The posterior position of merozoites nuclei in the present investigation were, also, detected in *C. rochalimai* (Paperna and Lainson, 2000). The presence of two to three nuclei in a meront (Paperna, 2007b) disagrees with the number of merozoites produced in a parasitophorous vacuole as described by the same author and others as well as the present study that necessitates the presence of two or more merozoites generations which was also confirmed by (Paperna, 2007b).

Gamonts of *C. schmidti* are similar to those described in *C. alloagamae; C. alloghyrae; C. heteronotis* and *C. pachydactyli* (Paperna and Landsberg, 1989b; Paperna, 2007b) from different reptilian hosts. Microgamonts have superficial small nuclei with homogenate nucleoplasm and without nucleoli, numerous dense inclusions, vacuoles and few amylopectin granules. Mature macrogamonts or macrogametes are filled with amylopectin granules and few wall-forming bodies 2. They have many canaliculi which may be characterstic for the genus *Choleoeimeria*. The addition of electron–dense materials to the unit membrane during macrogamonts growth was described in the present work. Degeneration of wall-forming bodies was also observed which was remarked in other *Choleoeimeria* species. The two types of wall-forming bodies appeared of the same electron density and wall-forming bodies 2 were not spongelike as those described from *Eimeria steidai* in the bile duct of rabbits (Mehlhorn, 2008).

The granular particles of the parasitophorous vacuoles in all *Choleoeimeria* species including the present one were rarely or not seen in other coccidian genera (Tse *et al*., 1986; Paperna and Ostrovska, 1989; Klein *et al*., 1992; Paperna and Lainson, 1999).

Taxonomic summary

Type host

Acanthodactylus schmidti (Reptilia: Lacertiidae).

Type locality

Al- Qassim deserts.

Prevalence

34,4 % (21/61).

Site of infection

Epithelial cells of gall bladder.

Pathology

Lizards appeared to be unaffected by the infection.

Type specimens

Oocysts in 10 % formalin and Phototypes are deposited at Laboratories of Parasitology, Faculty of Science and Arts, Al Rass, Qassim University.

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